

# Incubation Temperature for Total Bacterial Count of Frozen Sea Foods

P. R. G. VARMA, CYRIAC MATHEN, ANNAMMA MATHEW,  
FRANCIS THOMAS AND H. KRISHNA IYER

*Central Institute of Fisheries Technology, Cochin - 682 029*

Effect of incubation temperatures of 37°C ambient and 5–10°C on total plate count of commercial frozen prawns, squids, cuttle fish and froglegs were studied. Results indicate that incubation at ambient temperature gives the best results.

Total plate count (TPC) is generally recognised as an index of freshness, sanitary quality or both of frozen seafoods. TPC at 37°C is included as a criterion for quality assessment of frozen seafoods in the relevant Indian Standards. Our recent observation on the TPC of cooked-peeled-frozen prawns has shown 30°C (room temperature) to be a better incubation temperature than 37°C (Cyriac Mathen *et al.*, 1979). Hence it was deemed necessary to study the effect of incubation temperatures on TPC of frozen seafoods and the results are presented here.

## Materials and Methods

Ten samples each of the different varieties of commercially important frozen seafoods were drawn from the processing establishments at Cochin. Thus samples were taken from frozen prawns in headless (HL), peeled and deveined (PD), peeled and undeveined (PUD), peeled-deveined and cooked (PDC) and cooked and peeled (CP) forms and also from frozen froglegs, squid and cuttle fish. Plating was done in duplicates using tryptone glucose agar. Phosphate buffer was used as diluent. The plates were then incubated at 37°C for 48 hours, at room temperature for 72 h and at 5–10°C for 15 days. The results obtained were statistically analysed. Data were analysed using the analysis of variances technique. For the purpose of analysis the bacterial counts in each sample were converted to their common logarithmic values.

## Analysis of variance

**Table 1.** PD variety

Source	SS	df	ms	F
Total	12.6203	59		
Samples	6.5554	9	0.7284	16.41 <sup>c</sup>
Temperatures	3.9171	2	1.9586	14.11 <sup>c</sup>
Duplicates	0.0617	1	0.0617	1.39
Error	2.0861	47	0.0444	

**Table 2.** HL variety

Source	SS	df	ms	F
Total	19.8432	59		
Samples	3.7855	9	0.4206	2.11 <sup>a</sup>
Temperatures	6.6577	2	3.3289	16.71 <sup>c</sup>
Duplicates	0.0363	1	0.0363	0.18
Error	9.3637	47	0.1992	

**Table 3.** PUD variety

Source	SS	df	ms	F
Total	19.5528	59		
Samples	6.4649	9	0.7183	7.00 <sup>c</sup>
Temperatures	8.2486	2	4.1243	40.20 <sup>c</sup>
Duplicates	0.0151	1	0.015	0.15
Error	4.8242	47	0.1026	

**Table 4.** *PDC variety*

Source	SS	df	ms	F
Total	42.3498	59		
Samples	12.4512	9	1.3835	11.89 <sup>c</sup>
Tempera- tures	24.4299	2	12.2150	104.94 <sup>c</sup>
Duplicates	0.000039	1	0.000039	1
Error	5.4687	47	0.1164	

**Table 5.** *CP variety*

Source	SS	df	ms	F
Total	31.2506	59		
Samples	7.5087	9	0.8343	5.11 <sup>c</sup>
Tempera- tures	16.0585	2	8.0293	49.14 <sup>c</sup>
Duplicates	0.0017	1	0.0017	1
Error	7.6817	47	0.1634	

**Table 6.** *Froglegs*

Source	SS	df	ms	F
Total	24.3798	59		
Samples	18.9294	9	2.1033	36.64 <sup>c</sup>
Tempera- tures	2.7423	2	1.3712	23.89 <sup>c</sup>
Duplicates	0.0109	1	0.0109	0.19
Error	2.6972	47	0.0574	

**Table 7.** *Squid*

Source	SS	df	ms	F
Total	19.4500	65		
Samples	15.7917	10	1.5792	24.41 <sup>c</sup>
Tempera- tures	0.1734	2	0.0867	1.34
Duplicates	0.1230	1	0.0615	0.95
Error	3.3619	52	0.0647	

**Table 8.** *Cuttle fish*

Source	SS	df	ms	F
Total	32.0022	71		
Samples	26.0955	11	2.3723	34.13 <sup>c</sup>
Tempera- tures	1.9408	2	0.9704	13.96 <sup>c</sup>
Duplicates	0.0038	1	0.0038	0.05
Error	3.9621	57	0.0695	

a = significant at 5 % level

b = significant at 1% level

c = significant at 0.1% level

**Table 9.** *Summary of the results of statistical analysis*

Variety	Order of preference of incubation temperatures
PD	RT 5-10 37
HL	[50-10 RT] 37]
PUD	[37 RT] 5-10
CP	RT 37 5-10
PDC	[37 RT 5-10]
Froglegs	[5-10 RT] 37
Squid	RT [37=5=10]
Cuttle fish	RT 37 5-10

[ ] indicates that the values are not statistically significant.

### Results and Discussion

The results of statistical analysis are presented in Tables 1 and 9. Table 1 gives the analysis of variance of PD variety of prawn. It could be seen from Tables 1 and 9 that significantly high ( $p > 0.001$ ) bacterial count was observed at room temperature than at 37°C and 5-10°C. The count at 37°C was found to be the lowest among the three. In Table 2, the variation between bacterial count in HL variety was found to be significant at 0.1% level. From Table 9, it can be seen that the counts at room temperature and 5-10°C were higher compared to the count at 37°C. The count at 5-10°C was higher than the count at room temperature though the difference was not statistically significant. The results of analysis of variance of PUD variety in Table 3 and 9 shows that the counts at 37°C and at room temperature were significantly high ( $p > 0.001$ ). Among the two, the count at 37°C was higher compared to the count at room temperature, though the difference was not statistically significant. Table 4 gives the results for PDC variety. From Table 9, it can be seen that at 37°C and at room temperature the counts observed were significantly higher ( $p > 0.001$ ) compared to that at 5-10°C. Among the two, the higher count is observed at 37°C than at room temperature. The analysis of variance of CP variety showed that (Tables 5, 9), significantly high count was observed at room temperature ( $p > 0.001$ ). The frog-leg samples showed a significantly high

bacterial count ( $p > 0.001$ ) at room temperature and at 5–10°C (Tables 6 and 9). Among the two, the counts were high at 5–10°C (Tables 6 and 9). Regarding squid, there is no significant difference in bacterial count at the three temperatures (Table 7). But cuttle fish showed a significantly high bacterial count ( $p > 0.001$ ) at room temperature than at 37°C and 5 – 10°C (Tables 8 and 9).

From Table 9 incubation at room temperature is seen to be good in all the varieties of frozen seafoods studied, while incubation at 37°C and at 5–10°C was not good in some of the products. Hence RT

is preferred in routine examination of TPC of frozen seafoods. Though an additional time of 24 h is required to get the results, as usually tests for salmonella, which takes along with TPC, this additional time involved is not significant.

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#### Reference

- Cyriac Mathen, Annamma Mathew, P.R. Girija Varma & Francis Thomas (1979) *Fish. Technol.* 16, 33